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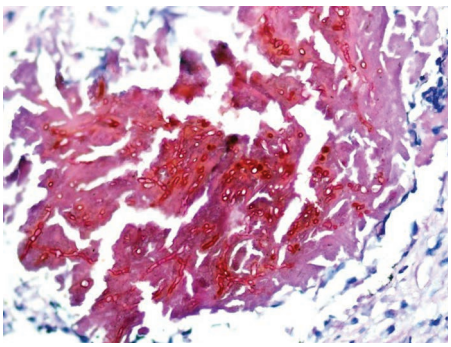
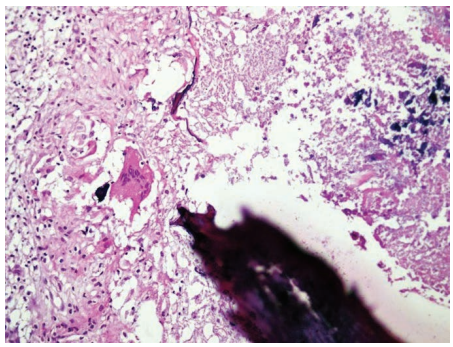
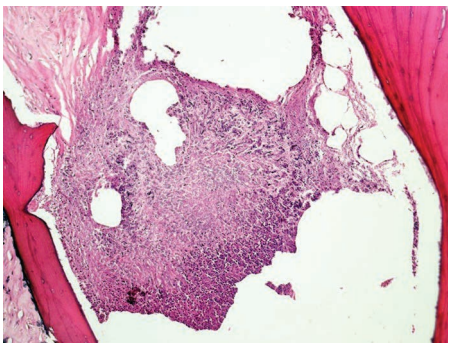
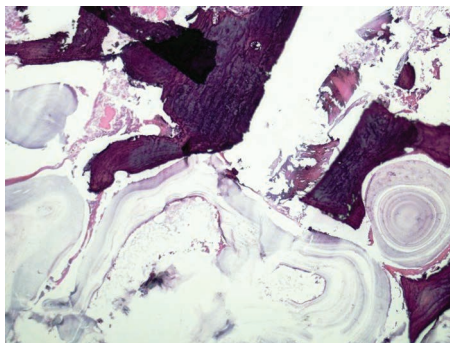
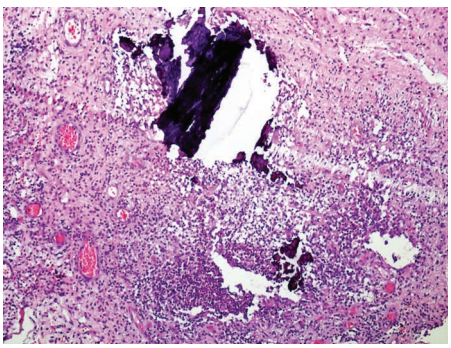
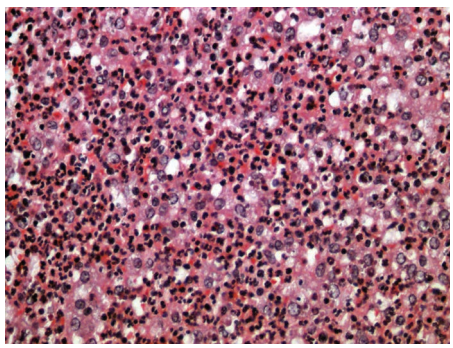
NEWSLETTER OF THE DEPARTMENTS OF PATHOLOGY & LABORATORY MEDICINE AND RADIOLOGY

LABRAD

MARCH 2017

VOL. 43, ISSUE 1

Infectious Diseases

آغا خان یونیورسٹی ہسپتال، کراچی
The Aga Khan University Hospital, Karachi

LABRAD

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From the Editor's Desk

LABRAD in 2017 begins on a thematic note. Our focus this time is "Infectious Diseases". Diagnostic modalities play a central role in the therapy of infectious diseases. With advancing technology, the main focus remains on rapid turnaround times. Diagnostic tests especially in our population should be performed cost effectively with rapid delivery of results in order to institute timely treatment. In some infections, early diagnosis and treatment prevent long-term complications or break the continuity of transmission of the infectious agent. In particular, diagnostic tests should come in handier when clinical symptoms are not specific for a particular infection (which occurs quite commonly). Apart from this, testing for asymptomatic infections, surveillance for hospital acquired infections, evaluating response to treatment, and detecting infections common to the developing world are pre-requisites for any diagnostic test employed for Infectious disease testing in Pakistan. In this issue, we have covered topics which include Chikungunya, Rubella, common bone and soft tissue infections, Epstein Barr virus infection and H.pylori to name a few. We have also added an interesting quiz for our readers. Hope you will enjoy the material and gain new insights in this particular field of Medicine.

Dr Natasha Ali
Editor, LABRAD

Chikungunya

Ms Najma Shaheen
Clinical Microbiology

Chikungunya is an arbovirus, belonging to the alphavirus family of bunyaviridae. It was first documented in Tanzania in 1953. Since then it has been reported from Europe (France and Italy), Africa (Madagascar, Kenya), Americas (United States, Mexico, and all of South America) and Asia (India, Sri Lanka, China, Malaysia, Thailand, Cambodia, and Yemen). Recently there has been an outbreak in Malir district of Karachi, Pakistan.

Due to its main symptom of severe joint pain, it was named chikungunya, meaning "that which bends up". Other symptoms include muscle pain, headache and rash is usually maculopapular, after the onset of fever.

Usually the disease is self-limiting and patient feels better within a week's time. However in some people joint pain may persist for several months. Certain people are at higher risk of severe disease with neurological or cardiac complications, like newborns or elderly, and those who have underlying comorbids, like heart disease, hypertension and diabetes.

Transmission

Chikungunya is transmitted to humans by the mosquito species *Aedes aegypti* and *A. albopictus*. Both mosquitoes have white strips on black bodies and legs and will be biting at any time of the day. *Aedes mosquito* is also responsible for dengue infection.

Figure 1: Aedes aegypti (left) and A. albopictus (right) (Courtesy: Google Images)

Diagnosis

- **Serology:** this diagnostic test includes the detection of IgM and IgG. These tests can be performed on serum or plasma using enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence, hemagglutination inhibition, or neutralization techniques.

IgM can be detected after the five days of infection and it remains detectable up to three months. The IgG antibodies can be detected in convalescent samples (Figure 2) and persist for years. The serologic tests are widely used because they are more cost-effective than other diagnostic tests in terms of disease detection. They are also easy to perform. It may show cross-reactivity with other alphaviruses and minimally with flaviviruses like dengue.

- **Culture:** Viral culture can detect virus within three days of illness. However, it requires skilled personnel and well-equipped facilities which may not be available everywhere.
- **Molecular diagnosis:** RT-PCR consider as the rapid diagnosis for Chikungunya infection as it can detect viral RNA in blood as early as five days after the symptoms appear. However, it will not detect the virus efficiently after five days of disease onset.

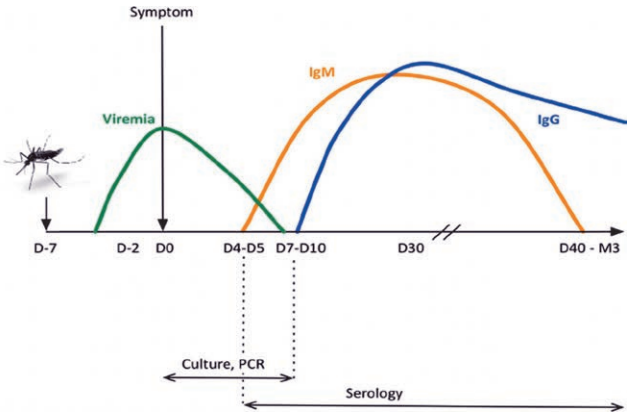

Figure 2: Contribution of PCR and blood tests on the diagnosis of Chikungunya, according to delay after test. [2]

Testing of Chikungunya IgM by Elisa Method at AKU

Chikungunya IgM Ab has been initiated in Clinical Microbiology lab by ELISA method. This test is performed twice a week.

Treatment and Prevention

- Good intake of fluid, taking rest and pain killers are the only treatment.
- Vaccination is not available as yet.
- Vector control is the main public health strategy. Draining stagnant water and keeping stored water covered will deplete the main sites of mosquito breeding. Mosquito coils, bed nets and mosquito repellents should be used to avoid mosquito bites.

Difference between Chikungunya and Dengue Virus:[1]

Character	Dengue	Chikungunya
Causative agent	Dengue virus, (Flavivirus of Flaviviridea family)	Chikungunya virus (alphavirus of Togaviridea family)
Vector	Aedes aegypti, A. albopictus	Aedes albopictus, A. aegypti
Incubation period	4-7 days	3-7days (range 1-12 days)
Disease duration	4-7 weeks	1-2 weeks, arthralgia may last for months
Symptoms	Fever, backache, large joint pain, headache, rash usually on face and limbs, retro-orbital pain. No arthritis	Fever, arthritis, pain in all joints, more prominent in small joints (worse in the morning), headache, eye pain, generalized rash
Hemorrhage and shock	Common in Dengue Haemorrhagic Fever (DHF) and Shock Syndrome (DSS)	Rare
Laboratory findings	Thrombocytopenia (<100,000/mm3), lymphopenia, and neutropenia. Elevated liver enzymes (ALT, AST); more with DHF	Mild thrombocytopenia (>100,000/ mm3), lymphopenia, elevated liver enzymes (ALT, AST)
Complications	Life threatening complications such as shock, ARDS, multi-organ failure and severe haemorrhage	Upto 10% develop chronic joint pain. Rarely neuroinvasive disease

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1. Microbiology notes by Pratiksha Pokhrei, updated on 31st March 2016. Last accessed 21st Jan 2017. http://www.microbiologynotes.com/differences-between-dengue-and-chikungunya/
2. Simon F. et al. French guidelines for the management of chikungunya (acute and persistent presentations). November 2014. Médecine et maladies infectieuses 45 (2015) 243–263

Rapid Molecular Diagnosis of Chlamydia Trachomatis (CT) and Neisseria Gonorrhoeae (NG) Urethritis

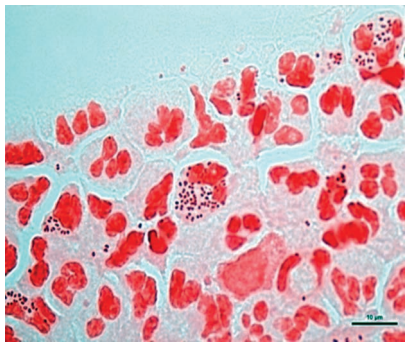
Ms Najma Shaheen
Clinical Microbiology

Among the list of microorganisms involved in sexually transmitted diseases, Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) are the most prevalent. Every year both CT and NG cause around 351.7 million cases of human infection1, associated with long term complications in both men and women especially pregnant women and their children. The complications include fetal growth retardation, preterm birth, low birth weight, pelvic inflammatory disease (PID) or ectopic pregnancy and infertility. It can also cause perinatal diseases such as conjunctivitis and pneumonia and sometimes even infant death. For mothers who have been infected with CT and NG there is also high incidence of vertical transmission of HIV from mother to children.

Neisseria Gonorrhoeae (NG)/Chlamydia Trachomatis (CT) Diagnosis

- **Microscopy**

Microscopic examination of urethral, endocervical swabs and secretions is a rapid test for the detection of NG in which they appear as intracellular gram negative bean shaped diplococci. Due to lack of specificity, gram stain is not recommended by the CDC for the detection of GC infection in asymptomatic men and women at any site other than the urethra.



Intracellular gram negative diplococci. Courtesy Google Images

Chlamydiae are non-motile bacteria that are located intracellularly and require giemsa stain for their detection. Giemsa stain is difficult to interpret and requires skilled personnel, and therefore is not readily available at all laboratories for chlamydia.

- **Culture**

Culture can be relatively inexpensive and is highly specific, but <100% sensitive when applied in routine clinical practice due to the fastidiousness of the ***Neisseria gonorrhoeae,*** which makes successful growth less likely if isolation methods, media, specimen transport are not optimal. Another disadvantage is that a minimum of 72 hours is required from specimen collection to the report of a presumptive culture.

Specimens that can be used for the detection of CT include endocervical swab, urethral swab, and female genital tract tissue collected during surgery. Culture has been the gold standard for Chlamydia in the past, but there are difficulties in maintaining the viability of organisms during transport and storage in the diverse settings. Therefore, non-culture tests are being used in diagnostic clinical laboratories.
The non-culture tests for ***C. trachomatis*** and ***N. gonorrhoeae*** include

— Enzyme immunoassays (EIAs)
— Direct fluorescent antibody (DFA) tests
— Nucleic acid amplification testing (NAAT)

Despite culture being the gold standard, the performance of Nucleic Acid Amplification Test (NAAT) is overall better because its sensitivity and specificity is higher for both CT and NG.

Rapid testing of CT/NG by Gene Xpert introduced at AKU

- **Technique:** This assay is a rapid real-time PCR nucleic acid amplification test. The cartridge-based assay detects DNA of *Chlamydia trachomatis* and *Neisseria gonorrhoeae.*
- **Turnaround time (TAT):** This test has been introduced in Clinical Microbiology Laboratory at Aga Khan University Hospital. At this point only urine samples are tested for CT/NG, while

testing on vaginal and endocervical specimens will be started later. Results are available within 90 minutes and it is performed 24 hours every day. It may be used as a point of care test if installed in clinics.

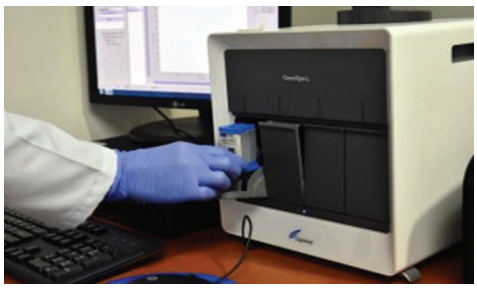


Figure 1: Courtesy: Google Images

Samples Required for Testing

- First-catch Urine for both males and females: Unpreserved (neat) urine or collected using the Urine Specimen Collection Kit provided by manufacturer may be used
- Endocervical /Vaginal swab specimens: Only the manufacturer-provided Vaginal/ Endocervical Specimen Collection Kit can be used for these specimens, as it preserves patient specimens during transport to the laboratory.

Overview of Rubella Testing

Iffat Arman
Section of Chemical Pathology

Rubella is a member of togaviridae family and humans remain the only natural host for this virus. Rubella is also known as German measles occurring throughout the world. Transmission is typically through inhalation of infectious aerosolized respiratory droplets and the incubation period following exposure can range from 12 to 23 days. The prodromal stage includes malaise and a low grade fever followed by characteristics of lymphadenitis and finally a macular or maculopapular rash. Primary infection during pregnancy can pass transplacentally to the fetus and can lead to fetal death or Congenital Rubella Syndrome (CRS). Babies born with CRS typically exhibit low birth weight, deafness, eye disease, mental retardation and cardiac abnormalities. Rubella infections are frequently mild or non-specific, making the infection difficult to diagnose clinically. For these reasons, pregnant women with an undiagnosed illness with rash should be evaluated for the possibility of an acute primary rubella infection. While the virus can be cultured in vitro, serology remains the principal means for establishing a clinical diagnose of acquired rubella and congenital infections.

Interpretation of Rubella antibodies Positive results

A primary infection induces an IgM and IgG response. Within four to six months, IgM levels becomes undetectable or very low. IgG decreases to low levels but lasts indefinitely and confers lifelong immunity. The secondary infection exhibits a rising IgG antibody without significant levels of IgM.

Rubella IgM Positive

Immunoglobin IgM serological testing is the primary diagnostic test use to confirm acute rubella infection. An IgM test should not be used to determine rubella immune status; IgM is used to diagnose acute and recent rubella infection. Rubella IgM testing should be performed on pregnant women who report symptoms of rubella or susceptible pregnant women who might have been exposed to rubella to rule out acute or recent infection.

Rubella IgG positive

A positive rubella IgG antibody test indicates rubella immunity (in pregnant females or vaccinated children). However the presence of rubella IgG in an infant after the decline of maternal antibodies (nine months of age) and the absence of vaccination or exposure to rubella will confirm CRS. Rubella IgG antibody avidity can be used to distinguish between recent infection and remote rubella infection, IgG increases with time; this is known as maturation of the immune response.

Biomarkers for Sepsis

Dr Sibtain Ahmed
Chemical Pathology

Sepsis refers to the presence of a serious infection that correlates with systemic and uncontrolled immune activation. To date no gold standard test is recognized for diagnosis of sepsis however microbiological culture is taken as a reference standard. Prompt diagnosis of sepsis is challenging, with turnaround times surpassing the limits for rapid diagnosis because microbiological culture results take several days to come up so it is common clinical practice to utilize available biomarkers that offer rapid kinetics leading to an improved patient care and facilitation. The various tests that are offered by our clinical laboratory include:

Serum C - reactive protein (CRP)

CRP is a protein produced in response to infection and it is extensively utilized in clinical practice to diagnose and manage patients with sepsis. It is a positive acute phase reactant whose synthesis in the liver is up regulated by IL-6. The mechanism of action in acute inflammation is that it may bind the phospholipid components of microorganisms, facilitating their removal by macrophages. Even though its low specificity may be its primary disadvantage as a biomarker of sepsis in adults, it is commonly used to screen for early onset sepsis in pediatrics. Normal concentration in healthy human serum is usually lower than 10 mg/L whereas in mild inflammation and viral infections it rises to 10–40 mg/L. Levels in active inflammation, bacterial infection are in range of 40- 200 mg/L. Whereas in severe bacterial infections and burns levels >200 mg/L have been recorded.

Serum Procalcitonin (PCT)

Procalcitonin is a pro hormone (peptide precursor) of calcitonin that is secreted by parenchymal cells, including hepatocytes, renal parenchymal cells, adipocytes, and muscle cells in response to bacterial toxins. Procalcitonin has also been used to distinguish fungal and viral infections from bacterial infections. During viral infections, PCT levels are reported to remain at low levels, often at concentrations found in healthy individuals. It follows a rapid kinetics with levels rising in two to four hours in response to bacterial infection. On the other hand its level doesn't show abrupt rise in patients with viral infections. It possesses a half-life of 22 to 26 hours, making it a superior marker in term of kinetics compared with CRP and other acute-phase reactants. However it faces certain limitation that includes possible elevation in noninfectious disorders, especially following trauma.

Furthermore PCT levels have been extensively used to guide empirical antibacterial therapy in patients with acute exacerbations of chronic bronchitis, community-acquired pneumonia, and sepsis. PCT also serves as a prognostic marker of sepsis as PCT levels have been found to be associated with increased mortality rates and correlate well with severity scores (APACHE, SOFA, and SAPS). The rapid elevation and sustainment of PCT levels in the serum during infection makes it an ideal biomarker. As a biomarker for bacterial infection, most studies find PCT to be a useful and accurate biomarker.

Plasma Lactate

Hyper-lactatemia is characteristically present in patients with severe sepsis or septic shock and may be ancillary to anaerobic metabolism owing to hypo perfusion or other multifaceted factors. The prognostic value of elevated blood lactate levels has been well recognized in septic shock patients, particularly if the high levels continue. Given the high risk for septic shock, all patients with elevated lactate >4 mmol/L (36 mg/dL) are considered the early goal-directed therapy zone irrespective of blood pressure. Mortality is high in septic patients with lactate ≥4 mmol/L making it a worthy prognostic marker as well.

Conclusion

Serum biomarkers discussed here have the potential to diagnose, monitor, stratify and predict outcome in sepsis. C-reactive protein is elevated in inflammatory and infectious conditions and has long been used as a biomarker indicating infection. Procalcitonin has more recently been shown to better distinguish infection from inflammation. Another important biomarker that has specific relevance to distinguishing sepsis from septic shock and predicting the prognosis of the latter is the lactate level.

Most Common Bone and Soft Tissue Infections Seen in our Practice

Dr Nasir Ud Din and Dr Muhammad Usman Tariq
Histopathology

Introduction

Soft tissue and bone infections can be caused by either direct penetration of a pathogen or by hematogeneous spread of the pathogen from the initial site. In this article we shall discuss common bone and soft tissue infections seen in our practice.

Bacterial Infection of Bone

The most common form of infection of bone (pyogenic osteomyelitis) is caused by hematogenous spread of bacterial infection elsewhere in the body such as a urinary tract infection or pneumonia. Osteomyelitis also occurs after surgery, bone fracture after road traffic accident and open wound over bone in diabetics. In the initial stages of infection, bacteria multiply, setting up a localized inflammatory reaction and resulting in localized cell death. With time, the infection becomes demarcated by a rim of granulation tissue and new bone deposition.

Although no organisms are recovered in up to 50 per cent of cases, when one is isolated, Staphylococcus aureus is by far the most common agent accounting for 80-90 per cent of all infections. Escherichia coli, Klebsiella and Pseudomonas species are associated with osteomyelitis in intravenous drug users and genitourinary tract infection. Salmonella is involved in osteomyelitis of patients with sickle cell disease. Haemophilus influenza and group B streptococci are involved in osteomyelitis of neonates.

In children, osteomyelitis usually affects the long bones of extremities, including the femur and humerus. Adults usually develop osteomyelitis of vertebrae, fractured long bones and at hip replacement. Osteomyelitis initially develops rapidly and is usually accompanied by pain, fever, and joint stiffness. Chronic osteomyelitis progresses slowly and may be the result of a previous bone infection. The histologic features of chronic infection also include infarction of the bone with bony sclerosis, as well as a mixture of inflammatory cells consisting of macrophages, lymphocytes, plasma cells, and neutrophils.

Tuberculous Osteomyelitis

Tuberculous osteomyelitis is one of the most common causes of bone infection seen in our practice. The usual source of origin is from foci of active visceral disease that are in the initial stages of primary infection. The spine is affected in 40-50 per cent of cases of tuberculous osteomyelitis, and is followed in frequency by the hip and knee joint bones in 25 per cent and 20 per cent of cases, respectively. In the spine, 98 per cent of infections are centered in the anterior column and the involved level is usually located in the region of T6-L3, leading to the formation of a gibbus or kyphosis. The histologic features consist of the presence of granulomas composed of aggregates of epithelioid macrophages admixed with multinucleated giant cells including Langhans type giant cells (Figure 1). Areas of caseous necrosis within a variably inflammatory and frequently necrotizing background are often seen. Acid-fast bacilli which are typically beaded and bent and most often found either in the necrotic zone or in the giant cells, but can be seen anywhere in the granulomatous deposit on ZN stain.

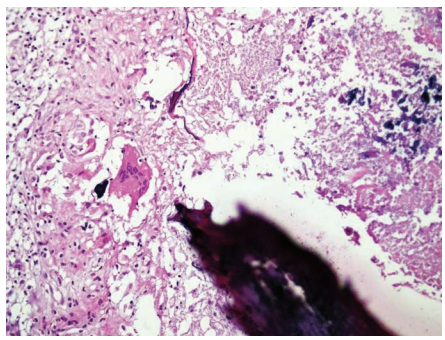


Figure 1: Granulomatous inflammation of bone with necrotizing granulomas formation

Parasitic Osteomyelitis

Human echinococcal infection occurs across the globe. It is more common in our population and results from the ingestion of contaminated water and food or handling live animals which act as definitive hosts such as dogs, or working with their pelts. The swallowed eggs hatch in the small intestine

and release minute hooked embryos which burrow through the bowel wall and are then transported by the bloodstream to various sites such as the lung, liver, brain, skeletal muscle, and eyes. Involvement of the skeletal system is rare and occurs in 0.2-4 per cent of cases. Once the organisms reach their final destination, they produce hydatid larval cysts. These unilocular cysts are filled with fluid and lined by an inner germinal membrane that produces brood capsules (Figure 2). The inner wall of the brood capsules facilitates an asexual budding process that generates thousands of new larval tapeworms (protoscolices) in daughter cysts. The hydatid cysts can achieve large size (2 to 30 cm), have a thick wall and contain clear or pale yellow fluid. The sites of bone disease commonly include the spine, pelvis and long bones. Once lodged within bone the hydatid cyst fills the spaces between the bony trabeculae and eventually results in their resorption and that of the neighboring cortex such that it extends into the adjacent soft tissues.

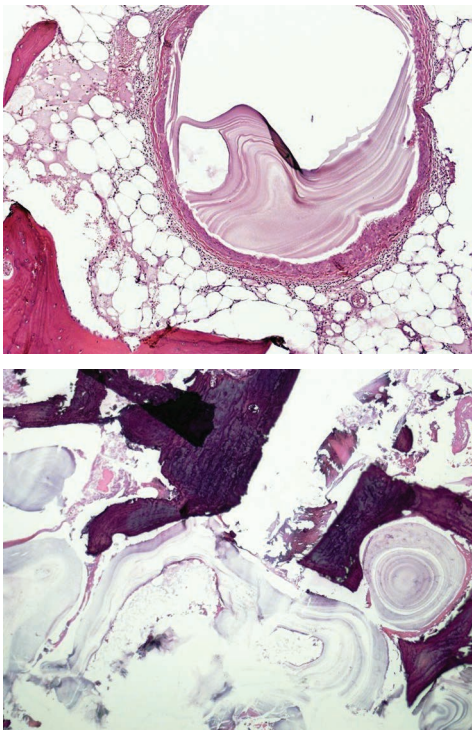


Figure 2: Hydatid cyst of bone. The acellular lamellated membranes are filling the intertrabecular areas.

Eumycetoma (Fungal mycetoma)

Mycetoma (named because of the tumour-like mass it forms) is a chronic granulomatous disease predominantly involving feet (hence also named Madura foot) and rarely can involve the hands, back or shoulders. It is characterized by localized infection of cutaneous and subcutaneous tissues by actinomycetes or fungi. Approximately 40 per cent of mycetomas worldwide are eumycotic as opposed to actinomycotic (i.e, caused by bacterial actinomycetes). The disease is marked by progressive destruction of soft tissue and nearby anatomic structures.

Mode of infection is an inoculation of the causative microorganism via small injuries of the skin. The clinical correlate of both forms of mycetoma is tumescence with abscesses, painless nodules, sinuses and discharge. The latter is commonly serous-purulent and contains grains (filamentous granules) which can be expressed for diagnostic purposes. Distinctive for both eumycetoma and actinomycetoma, are the formation of grains. Grains represent microcolonies of the microorganism in vivo in the vital tissue. Differentiation between actinomycetoma and eumycetoma is important because they respond to different treatments. The diagnosis can be established only by finding the “sulfar granules”. The granules of both eumycetoma and actinomycetoma stain with PAS and silver stain; those of eumycetoma are composed of septate hyphae (4-5 µm), whereas granules of actinomycetoma usually consists of fine, branching filaments of bacillary

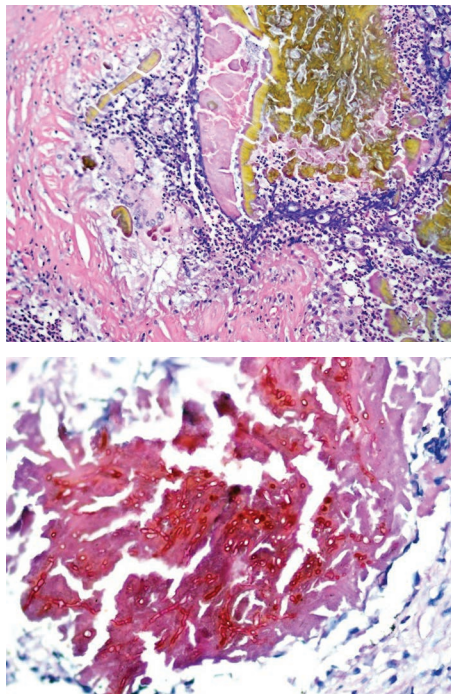


Figure 3: Fungal mycetoma. There is massive tissue reaction with epithelioid cell granulomas together with grain which is a conglomerate of septated and branched hyphae. Staining: PAS (Periodic-acid-Schiff) with diastase reaction.

forms (1 µm). Filaments of actinomycetoma are gram positive, whereas the hyphae in grains of eumycetoma are gram negative.

Fungal Osteomyelitis

Fungal osteomyelitis may occur as a component of a multisystem infection or as an isolated condition. It may evolve slowly and masquerade other conditions such as tuberculosis, myeloma, and metastatic disease. Fungal osteomyelitis is a serious illness and is most virulent in immunodeficient patients. Bone infection occurs by hematogenous dissemination, direct inoculation, and spread from a neighboring site of disease. The clinical presentation and outcome varies according to the specific pathogen, location of the infection, and host factors. Invariably the infection causes pain, localized swelling, bone destruction with reactive changes, and a prominent inflammatory reaction. Definitive diagnosis usually requires identification of the organism in tissue specimens or cultures (Figure 4).

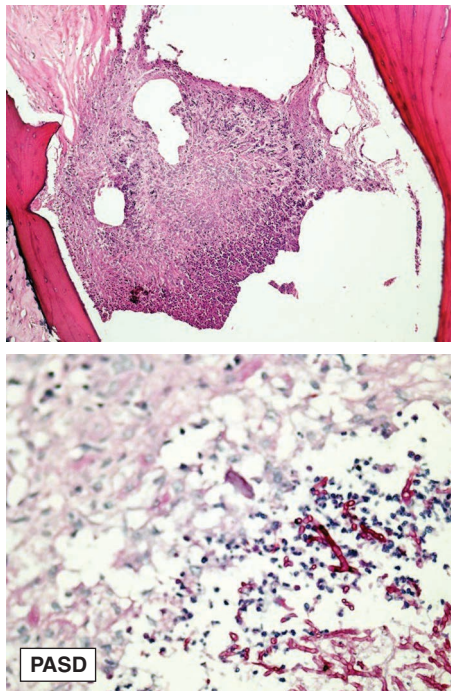


Figure 4: Fungal infection of bone. Numerous branching hyphae are present in necrotic background which is positive for PASD stain.

Overview of Epstein - Barr Virus Antibodies Panel

Dr Tayyaba Hassan
Chemical Pathology

Epstein-Barr virus (EBV), also known as human herpesvirus four is a member of the herpes virus group and is the etiologic agent of infectious mononucleosis. Other disorders due to EBV infection include Burkitt lymphoma, nasopharyngeal carcinoma and lymphoproliferative syndromes especially in patients who have undergone renal or bone marrow transplantation or those with AIDS. Most people have been exposed to EBV by early adulthood. Many people become infected with EBV in childhood. EBV infections in children usually do not cause symptoms, or the symptoms are not distinguishable from other mild, brief childhood illnesses. People, who get symptoms from EBV infection, usually teenagers or adults, get better in two to four weeks. However, some people may feel fatigued for several weeks or even months. Symptoms of EBV infection can include fever, inflamed throat, swollen lymph nodes in the neck, enlarged spleen, inflamed liver or rash. After you get an EBV infection, the virus becomes latent (inactive) in your body. In some cases, the virus may reactivate. This does not always cause symptoms, but people with weakened immune systems are more likely to develop symptoms if EBV reactivates. EBV is difficult to diagnose using one serological assay.

The EBV virus is composed of a double helix of DNA. The DNA is surrounded by a protein nucleocapsid (Figure 1). This nucleocapsid is surrounded by a tegument made of protein, which in turn is surrounded by an envelope containing both lipids and surface projections of glycoproteins which are essential to infection of the host cell.

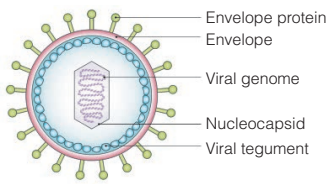


Figure 1: EBV structure

Diagnosis of an acute/ recurrent infection is best accomplished by using a profile or panel of assays which has three components:

- IgG Viral capsid antigen (VCA)
- IgM VCA
- IgG Epstein Barr nuclear antigen (EBNA)

The presence of VCA IgG antibodies indicates infection sometime in the past. Antibodies to EBNA develop six to eight weeks after primary infection and are detectable for life. Specimens drawn too early during the course of the disease may not contain detectable antibody to Epstein-Barr virus (EBV). Another specimen drawn one to two weeks later may be required.

Table 1: Interpretation of EBV antibody profile

	EBNA-IgG	VCA-IgM	VCA-IgG
No previous exposure	Negative	Negative	Negative
Early primary infections	Negative	Positive	Negative
Acute infection	Negative	Positive	Positive
Past infection	Positive	Negative	Positive
Recovery stage	Positive	Positive	Positive

Diagnosis of Helicobacter Pylori Infection in Developing Countries- Pros & Cons

Ms Syeda Kiran Zaidi
Clinical Microbiology

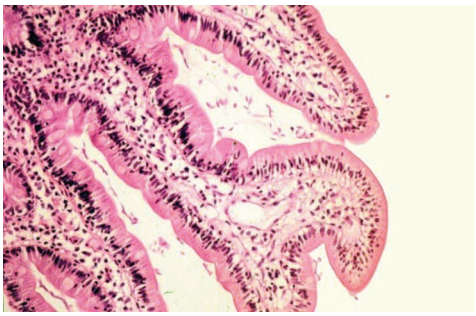
Helicobacter pylori infection is now recognized as a worldwide problem. It is the most common cause of chronic gastritis, peptic ulcer and it can also lead to gastric cancer. Like all developing countries, Pakistan also has high H. pylori prevalence, >85 percent (1). The most common route of H.pylori infection is fecal- oral. Mostly, patients infected with H.pylori are asymptomatic. When signs and/or symptoms are present, they may include nausea, vomiting, abdominal pain, heartburn, diarrhea, hunger pangs in the morning, and halitosis (bad breath). Diagnosis of H.pylori infection is divided into two subgroups i.e. invasive and non-invasive tests. Invasive tests include endoscopy, histopathology, culturing from tissue and rapid urease test, whereas non-invasive tests include stool antigen test, antibody response as a marker of disease, and urea breath test.

Non-Invasive Tests

Stool antigen test detects active H.pylori infection, independent of age, and has high sensitivity and specificity; whereas, Anti-H. pylori IgG is a supportive test as antibody titers remain high for months or years even after treatment (Table 1). For optimal results of stool antigen test, the patient should be off antacids and proton pump inhibitors (PPIs) for at least two weeks. Serological testing is relatively inexpensive and it is widely available. In a low-prevalence area a negative test has more value than a positive test while in a high-prevalence area, positive serology can reasonably be accepted as positive. Urease breath test is also used as a common non-invasive test; it is highly sensitive and specific. One of the limitations is the presence of other urease producing bacteria in the stomach, e.g. H. heilmannii, which might lead to false positive results. Moreover, acute bleeding and co-medication can lead to false negative test results.

Invasive Tests

Among invasive test for H. pylori endoscopic biopsy for histology remains the gold standard and has a sensitivity of 95 percent and specificity of 100



H. pylori seen on H&E. Image courtesy
http://www.pathologyoutlines.com/caseofweek/case157.htm

Alert: WHO has identified Helicobacter pylori as one of the 12 priority organisms (Priority 2 HIGH) for anti-microbial resistance, i.e. resistant to clarithromycin

percent but sensitivity may be reduced by the use of PPIs, antibiotics, bismuth containing compounds and test is also time-consuming and difficult to obtain. H.pylori culture is not a routine procedure because isolation of this microaerophilic organism from gastric biopsy specimens takes five–seven days and has an extremely low yield. Rapid urease test on antral biopsy provide a simple, rapid, and cost-effective method for the detection of H. pylori. However, the value of these tests depends not only on performance, but also on rapidity, making them a practical tool for the endoscopist in decision-making as to whether or not therapy should be prescribed. False negative results can occur in patients taking antisecretory drugs.

Table 1: Comparison of diagnostic tests for Helicobacter pylori infection

Test	Sensitivity	Specificity	Positive Predictive Value	Comments
Stool Antigen test	96%	95%	84%	• Non-invasive and inexpensive. • Highly sensitive and specific before and after treatment
Serology (IgG Ab)	85-92%	79-83%	64%	• Highly sensitive but does not accurately identify active infection • Not recommended after therapy
Urea Breath test	95%	96%	88%	• Preferred test for confirming eradication • Not to be performed within 4 weeks of antibiotic therapy • Performed in clinic; variable availability
Biopsy	>95%	>95%		• Gold Standard. Detection is improved by using special stains for e.g. the Warthin Starry silver stain, or Giemsa staining
Culture	~30-40%			• Highly specific; poor sensitivity if adequate transport media are not available • Expensive due to expertise and special culture requirements, which may not be available
Rapid Urease test	>98%	99%	99%	• Rapid and cheap. Post-treatment sensitivity reduced.

Aga Khan University Hospital Clinical Laboratories offer *H. pylori* stool antigen, Anti-*H. Pylori* IgG and histopathology for diagnosis of this disease. However, there is a need to introduce rapid molecular tests for better diagnosis of *H. pylori* infection and antibiotic resistance.

Screening for Neonatal Infections with TORCH Profile

Ms Nasreen Bano
Chemical Pathology

The TORCH profile is a group of tests used to screen newborns and, sometimes, pregnant women for certain infections that can cause birth defects in a baby if the mother contracts them during the pregnancy. The original concept of the TORCH perinatal infections was to group five infections with similar presentations, including rash and ocular findings A TORCH profile can detect numerous diseases, including herpes simplex, rubella, cytomegalovirus and toxoplasmosis (Table 1).
A TORCH screen is typically performed when a woman shows symptoms of any of these diseases during pregnancy.
The profile aids in the diagnosis of both congenital and acute acquired toxoplasmosis, cytomegalovirus, rubella and herpes simplex virus by testing IgM antibodies of all mentioned infections. The presence of IgM antibodies indicates acute or recent infection.

Table 1: Clinical manifestations of congenital infections

Infection	Clinical Manifestations suggestive of specific congenital infections in the neonate
Toxoplasmosis	The most common finding is chorioretinitis Hydrocephalus Intellectual disability (mental retardation) Intracranial calcifications (diffuse) Otherwise unexplained mononuclear CSF pleocytosis or elevated CSF protein Classic triad of congenital toxoplasmosis consists of chorioretinitis, hydrocephalus, and intracranial calcifications
Rubella Virus Infection (German Measles)	Hearing impairment(Sensorineural hearing loss) Cataracts, congenital glaucoma, pigmentary retinopathy Cardiac defects (most commonly patent ductus arteriosus or peripheral pulmonary artery stenosis) Radiolucent bone disease
Cytomegalo virus (CMV) Infection	Microcephaly Hepatoslpenomegaly Periventricular intracranial calcifications Thrombocytopenia Sensorineural hearing loss Preterm born infants infected with CMV after birth (especially via breastmilk) can have cognitive and motor impairments later in life.
Herpes Simplex virus (HSV) Infection	Most are asymptomatic at birth. Mucocutaneous vesicles or scarring Elevated transaminases Conjunctivitis or keratoconjunctiitis CSF pleocytosis. 3 patterns of ~ equal frequency with symptoms between birth and 4wks: Localized to the skin, eyes, mouth (SEM) Localized CNS disease Disseminated disease (present earliest)

Blood and Bone Marrow Changes in Viral Infections

Dr Muhammad Shariq Shaikh
Haematology

Bone marrow is the primary site of haemopoiesis throughout the life of an individual. A variety of infections can involve bone marrow however response of the bone marrow to infection is highly variable, depending on presence of co-existing disease, the age of the patient, nature (bacterial, viral, parasitic or fungal) and duration of the infection. In this article blood and bone marrow changes in viral infections will be discussed.

Viral infections are usually associated with lymphocytosis that sometimes exhibit atypical features (Figure. 1). Infectious mononucleosis caused by the Epstein-Barr virus (EBV)

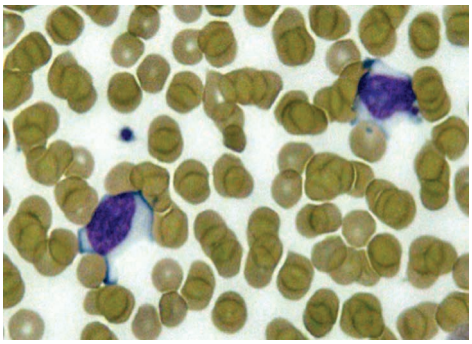


Figure 1. Atypical Lymphocytes on Peripheral blood film (at 40x).

infection is characterized by the production of large numbers of atypical lymphocytes. These lymphocytes are characteristically pleomorphic, have abundant basophilic cytoplasm and prominent nucleoli. The similar picture may also be a feature of other viral infections like Cytomegalovirus (CMV) and hepatitis A as well as toxoplasmosis and some hypersensitivity reactions to drugs. In contrast, viruses that cause lymphocytosis without significant atypical features include Coxsackie virus, various adenoviruses and Human immunodeficiency virus (HIV). Haemoglobin concentration may be reduced due to bone marrow suppression, haemorrhage or haemolysis; conversely it may sometimes be increased in patients with a severe capillary leak syndrome in certain viral infections. A wide range of viral haemorrhagic fevers are associated characteristically with thrombocytopenia primarily due to increased platelet consumption. Immune mechanism in causation of cytopenias is well-known, as in infections with Rubella, EBV, and Chronic hepatitis C infections.

In addition to the features mentioned above, bone marrow cytology in some infections particularly by herpes viruses reveals haemophagocytosis. In HIV-positive patients, Human Herpes Virus 8 (HHV8) infection may cause multicentric Castleman's disease. Another non-specific feature is reactive increase in T-lymphocytes and plasma cells. Bone marrow hypocellularity with suppression of one or more lineages may be seen in several viral infections including CMV, HHV8, EBV, and Dengue fever. Erythroid lineage may be hyperplastic with haemolysis as in infectious mononucleosis whereas; in parvovirus-induced pure red cell aplasia there are prominent, very large pro-erythroblasts with a striking lack of more mature cells. Rarely, in immunologically competent people, persistent infection can lead to chronic red cell aplasia. In cases where thrombocytopenia is due to increased platelet destruction, bone marrow shows normal or increased number of megakaryocytes. Rarely, platelets are visible in bone marrow macrophages.

Bone marrow histology provides better assessment of cellularity as well as certain findings that may not be recognized on examination of aspirate. CMV infections may depict eosinophilic intranuclear inclusions, albeit rarely these inclusions are either present within granulomas or interspersed in between haemopoietic cells. In acute EBV infection, bone marrow may exhibit gelatinous transformation or a diffuse increase in reticulin. Reactive lymphoid nodules in viral infection may be seen fairly commonly. Examples include chronic hepatitis B, hepatitis C and HIV infections. Where available, the presence of various viruses can be confirmed by immunohistochemistry using monoclonal antibodies. Lastly, viruses have also been implicated in causing lymphoproliferative disorders; chronic hepatitis C infection

can be complicated by the development of low grade B-cell lymphoma. Whereas, in patients with impaired immunity and X-linked lymphoproliferative disorder, EBV infection has been found associated with a spectrum of lymphoproliferative disorders, including lymphoma.

The peripheral blood and bone marrow response to viral infections are largely non-specific and similar changes occur in many other conditions, including trauma, administration of growth factors, carcinoma, Hodgkin and non-Hodgkin lymphoma and autoimmune disorders such as systemic lupus erythematosus. Therefore, high index of suspicion along with careful morphologic examination of both bone marrow aspirate and trephine biopsy is required in patients with suspected viral infection.

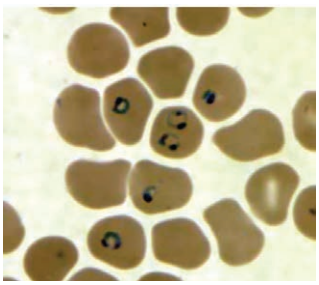
Malaria

Dr Natasha Ali

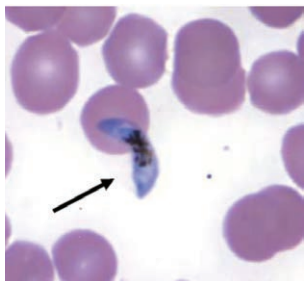
Malaria is a parasitic infection transmitted through the bite of infected female Anopheles mosquito. It is a preventable and curable disease and many countries are involved in the malaria eradication programme. Sub-Saharan Africa has the highest burden of disease. In 2015, 90 percent of malarial infection and 92 percent of deaths related to malaria occurred here. In Pakistan, the malaria transmission period occurs post monsoon but sporadic cases occur throughout the year.

Anopheles mosquitos are called “malaria vectors”. There are, in total five species of malaria that cause infection. Four of these cause infection in humans while one is responsible for zoonotic infection. These species are:

1. ***Plasmodium Falciparum:*** Found in tropical and subtropical areas (e.g. Africa). *P. falciparum* is responsible for complicated and cerebral malaria. A parasitemia of more than two percent requires red blood cell exchange along with anti-malarials as part of treatment. In the peripheral blood smear following forms are seen routinely:

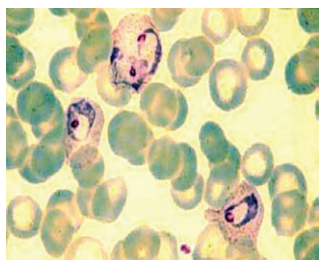


Trophozoites: thin delicate rings in blood

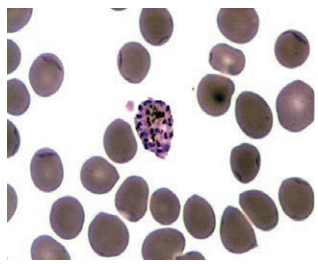


Gametocyte showing presence of “Laveran’s bib”

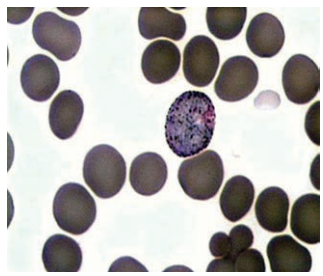
2. ***Plasmodium vivax:*** found mostly in Asia, Latin America and few parts of Africa. It is largely absent in West Africa where individuals lack Duffy red blood cell antigen – strong predictor of p.vivax red cell invasion. Based on its distribution, it is the most prevalent human malarial parasite. Glucose-6-phosphate dehydrogenase deficiency is associated with protection and decreased parasite density in p.vivax infections. P.vivax has a very strong predilection to infect reticulocytes. It can become latent in the liver, the stage known as hypnozoites responsible for causing relapses after months or years from the infection. The stages commonly seen in peripheral smear are:



Trophozoite (p.vivax)

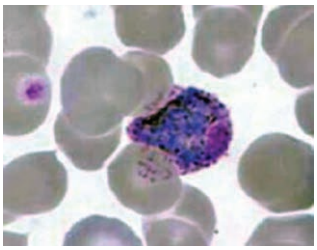


Schizont (p.vivax)

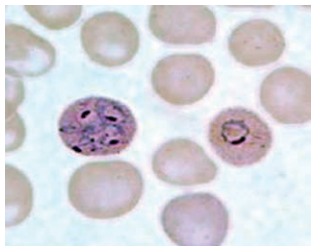


Gametocyte (p.vivax)

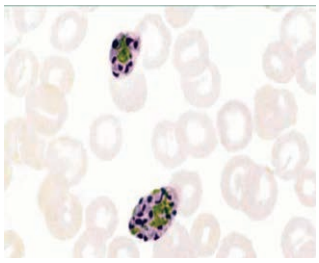
3. ***Plasmodium ovale:*** Distributed primarily throughout Sub-Saharan Africa. It has also been reported from various islands of Western Pacific. The diagnosis is based on its characteristics on blood film and differentiation from *p.vivax.* As *p.vivax, p.ovale* also has a hypnozoite stage where the parasites become suspended for many months. After the sporozoites are introduced through the bite of infected mosquitoes, these forms invade the liver and within a single parenchymal cell, the parasite matures within ten days. Eventually, hundreds of merozoites are produced which invade reticulocytes and initiate the erythrocytic cycle. In the blood smear, following stages are observed:



Gametocyte (p.ovale)

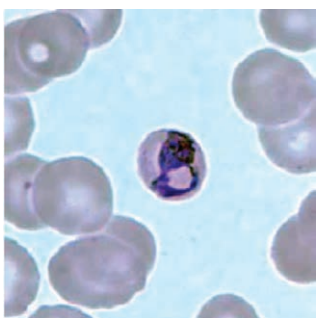


Trophozoite (p.ovale)

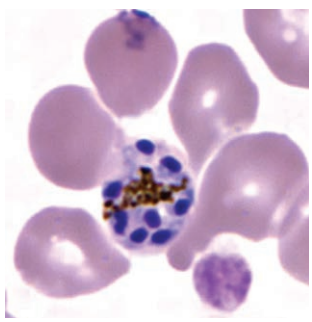


Schizont (p.ovale)

4. ***Plasmodium malariae:*** Infections caused by p.malariae coincide with those caused by p.falciparum. It is widely distributed in Sub-Saharan Africa, Southeast Asia and islands of Western Pacific. Cases have also been reported in the Amazon basin of South America. Not just limited to mosquitoes and humans, scientists at Osaka University in Japan have discovered strains of p.malariae in chimpanzees from Africa. Despite being infected, the chimps have not shown symptoms of disease. The stages identified on peripheral blood smear include:

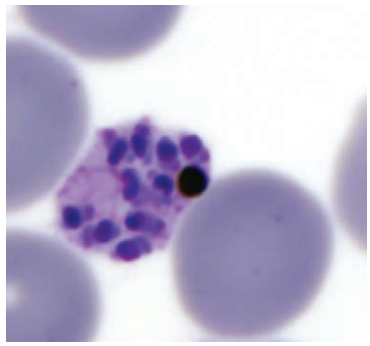


Trophozoite (p.malariae) – "Basket form"

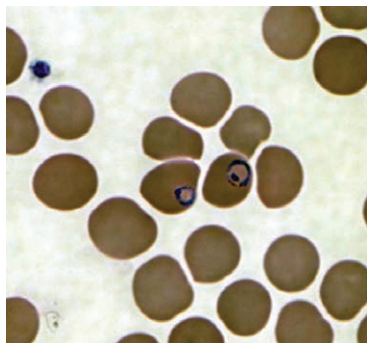


Gametocyte (p.malariae) – rosette pattern

5. ***Plasmodium Knowlesi:*** Traditionally was regarded as a rare disease, occurring sporadically in humans. However, recent findings of a large number of infected patients in Malaysian Borneo; reports of human cases in Thailand, Myanmar, the Philippines, Singapore; and few reports of p. knowlesi malaria in travelers to the Malaysian Borneo suggest that p. knowlesi may be more widespread among humans than perceived. The infection occurs in long-tailed and pig-tailed macaques that are present in forested areas of Southeast Asia. P. knowlesi can be transmitted from monkeys to humans by the bite of an infected mosquito. In the blood smear, the parasite is identified by:



Schizonts (p.knowlesi)



Trophozoites (p.knowlesi)

The widely distributed malaria species causing infection in Pakistan include p.falciparum and p.vivax. Epidemiologically, Pakistan is classified as a moderate malaria endemic country. P.vivax is the major parasite species responsible for causing more than 80 percent of the confirmed, reported cases in the country.

Film Array Multiplex PCR: A Syndromic Diagnostic Approach

Dr Mohammad Zeeshan
Clinical Microbiology

Introduction

Early and reliable diagnosis of infectious diseases is increasingly challenging due to changing epidemiology in underlying diseases and use of drugs affecting the immune system. Therefore, throughout the globe, a paradigm shift has been observed from conventional time-consuming, laborious, and observer dependent tests towards molecular diagnostics.

The role of microbiological evidence in infectious disease diagnosis is vital in initiating the appropriate antimicrobials, deciding patients' prognoses, infection control and prevention strategies, and duration of hospital stay. Conventional tests such as microscopy and culture for bacteria and fungus have limitations due to low microbial count and longer incubation time. Limited viral testing in routine clinical laboratory often leads to under-diagnosis. Sometimes chemical analysis of sample (e.g. CSF) is not always reliable in delineating the causative agents, rendering inconclusive especially after the use of antibiotics.

Moreover, diversity of causative agents creates a predicament for physicians due to overlapping clinical findings. They face a dilemma of deciding which test to order since a single diagnostic platform is not usually available for a particular presentation. This results in under diagnosis of infections and compromised patient care.

A solution to this problem is molecular syndromic diagnostic approach which uses broad test menu on a small amount of a single sample. Film Array, an FDA-cleared multiplex PCR system, is one such platform approved for clinical use.

Principle

Multiplex PCR is a molecular biology technique in which more than one target sequence can be amplified by using multiple primer pairs in a reaction mixture. The system is designed to be

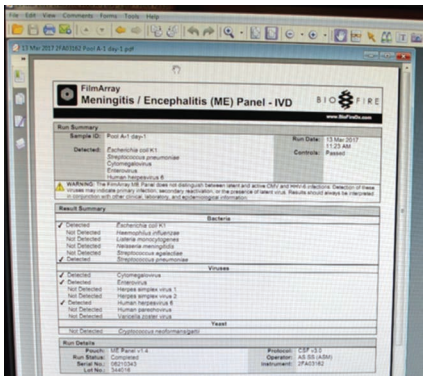
used with comprehensive panels for different syndromes. Each panel offers testing for sets of causative organisms including bacteria, viruses, yeast, parasites and/or antimicrobial resistant genes in one go, with turnaround time of a little over an hour. The following panels are available which include the most common clinical challenges these days:

- **Meningitis \ Encephalitis Panel (ME):** For 14 most relevant ME-associated pathogens, including bacteria, viruses and a yeast (See Table).
- **Respiratory Panel:** For a panel of 17 viruses most commonly implicated in both upper and lower respiratory tract infections, and three bacteria which cannot be recovered on routine culture: Bordetella pertussis, Mycoplasma pneumoniae and Chlamydophila pneumoniae.
- **Blood Culture Identification Panel:** For a comprehensive list of 24 pathogens and three antibiotic resistance genes associated with bloodstream infections.
- **Gastrointestinal Panel:** tests for 22 common gastrointestinal pathogens including viruses, bacteria and protozoa that cause infectious diarrhea.

The Clinical Laboratory of Aga Khan University Hospital microbiology section plans to introduce the Meningitis and Encephalitis panel (MEP) on Film Array based testing of CSF samples for patients with suspected meningitis and/or encephalitis.

	Meningitis\Encephalitis		
MICROORGANISMS	**Bacteria:** — Escherichia coli K1 — Haemophilus influenzae — Listeria monocytogenes — Neisseria meningitidis — Streptococcus agalactiae — Streptococcus pneumoniae	**Viruses:** — Cytomegalovirus (CMV) — Enterovirus — Herpes simplex virus 1 (HSV-1) — Herpes simplex virus 2 (HSV-2) — Human Herpes virus 6 (HHV-6) — Human Parecho virus — Varicella zoster virus (VZV)	**Yeast:** — Cryptococcus neoformans/gattii
Sample type	CSF		
Sample volume	200 µl		
Hands-on time	2 minutes		
Run time	1 hour		

How it works



The film-array reagent pouch stores all the necessary reagents used in sample preparation, reverse transcription and amplification. The hydrating solution and sample are added to the pouch which is then loaded into the film array machine. The results are analysed automatically and displayed on the monitor as soon as the run is completed in the form of a list of all the organisms "detected" and "not detected" in the panel.

Advantages

1. It has high sensitivity and specificity for each of the organisms tested in the panel.
2. No precise measurement or pipetting, therefore, no extensive hands-on technical training is required in the entire procedure; its use in Point of Care Testing (POCT) is increasing.
3. Rapid identification of microorganism in one-go with turn-around-time of one hour.
4. It is a closed system, minimizing chances of contamination.
5. Although its one-time cost is high, rapidity and reliability of test leads to shorter hospital stay and lessen unnecessary procedures, demonstrating its overall cost-effectiveness.
6. Identification of targeted organisms also minimizes the use of inappropriate antibiotics and other drugs, preventing emergence of antimicrobial resistance and aiding antimicrobial stewardship.

World Rare Disease Day

Dr Lena Jafri
Section of Chemical Pathology

Karachi, Mar 10, 2017: 'CME Seminar on World Rare Disease Day' was jointly organized by the Department of Pathology and Laboratory Medicine, Aga Khan University Hospital (AKUH) and Pakistan Society of Chemical Pathology (PSCP) at AKUH Karachi Pakistan. This CME seminar was arranged to mark the occasion of world rare disease day, which is observed worldwide on the last day of February each year. The objective was to create mass awareness about rare diseases and to ensure that patients with rare disease have access to treatment. We have been organizing this CME since the last two years. The theme for this year world rare disease day was research; with the slogan: 'with research, possibilities are limitless!'.

'For most of the rare diseases, the reality is that the answers to most basic questions are missing. It is only through research that identification of previously unknown diseases and understanding of diseases evolve. Research leads to the development of new innovative treatments and in some cases a cure.' said Dr Aysha Habib Khan, Associate Professor and Section Head, Chemical Pathology, AKUH while delivering the welcome address. She added that research is considered a key in bringing hope to the millions of people living with a rare disease across the world and their families. Dr Hafsa Majid Consultant Chemical Pathologist at AKUH gave an overview of rare diseases and highlighted the challenges faced in the diagnosis of these diseases in Pakistan. She shared the local data of Inherited Metabolic Diseases (IMD) diagnosed with locally available expertise at AKUH for organic acidurias & aminoacidopathies in high-risk Pakistani pediatric population. During the past few years many rare diseases have been reported with further additions to this never ending list, explained Dr Majid.

An enlightening talk on 'Clinical Application of Multiplex Ligation-dependent Probe Amplification ' was given by Dr Zeeshan Ansar, Assistant Professor at the section of Molecular Pathology AKUH. In comparison to PCR, MLPA amplifies multiple genes in one run. Additionally MLPA can detect carrier status of inherited disease. Dr Salman Kirmani, Associate Professor, Chair Department of Pediatrics and Child Health discussed the importance of chromosomal microarray for diagnosing rare disorders. He explained that using this latest technology, it is now possible for smaller and more complex chromosome defects to be identified. Dr Kirmani explained that this technology is able to identify numerical abnormality however it cannot detect structural defects or anomalies easily.

Panel of speakers in the question answer session at 'CME Seminar World Rare Disease Day'

Dr Lena Jafri, Assistant Professor Consultant Chemical Pathologist at AKUH emphasized the importance of College of American Pathologists (CAP) proficiency testing surveys as an educational and learning tool in her talk 'Competency Based Educational Challenges of Biochemical Genetics Laboratory'. She discussed few cases from CAP surveys and highlighted its importance in reliability and validity of patient reporting. 'Inherited Metabolic Disorders In Pakistan-reaching the unreached' was an interesting talk by Dr Bushra Afroze, Consultant Pediatrician & Clinical Geneticist at AKUH, who specializes in treating IMD. She introduced the audience to her new venture of tele health clinic between two clinical locations in two different cities of the country. With real life cases she explained how from a distance she has been managing patients with rare diseases. She emphasized that a strong liaison between clinicians in a country like Pakistan with limited

clinical expertise in IMD can be a pragmatic approach to provide services to more patients at their doorsteps.

Dr Farooq Ghani Associate Professor and Service Line Chief Department of Pathology and Laboratory Medicine in the closing remarks appreciated the team work of pathologists and pediatricians at AKUH in putting together this CME seminar and in helping the children and families with rare disease. We know that there is a dearth of awareness regarding these rare diseases and the need of the hour is to spread knowledge about them in order to prepare ourselves in such a way so that we can limit the dreadful effects of such diseases. This CME was an attempt in this regard with eminent speakers from Pathology & Pediatrics.

Radiology Pathology Correlation

Dr Nasir Ud Din and Dr Dawar Khan
Histopathology and Radiology

A 15 year old boy presented with moderate localized nocturnal pain of right distal thigh which was relieved by aspirin. A plain radiograph showed an oval shape lytic lesion along the long axis of the bone surrounded by a thick dense rim of reactive sclerosis that fades imperceptibly into surrounding bone (Figure 1A). On lateral view, periosteal new bone formation is noted (Figure 1B).

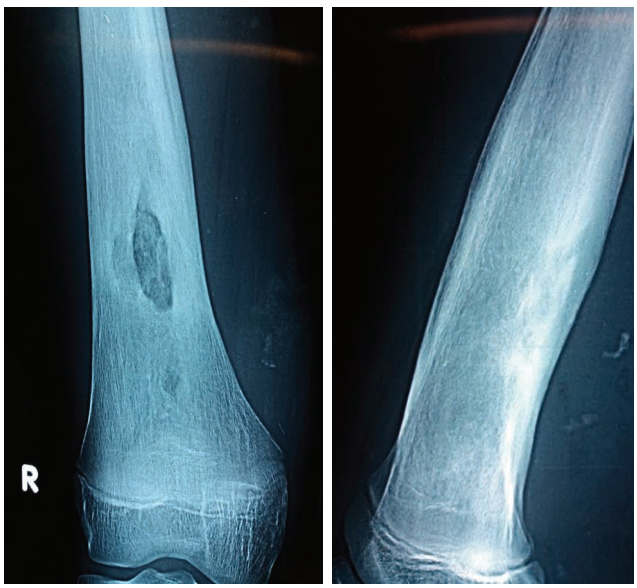


Figure 1A,B. AP and Lateral view of distal femur shows lytic lesion posteriorly with sclerotic margins and thick periosteal reaction.

A biopsy is done and histological examination revealed neutrophilic abscess along with granulation tissue formation (Figure 2A,B). The final diagnosis after radiological-pathological correlation was Brodie abscess.

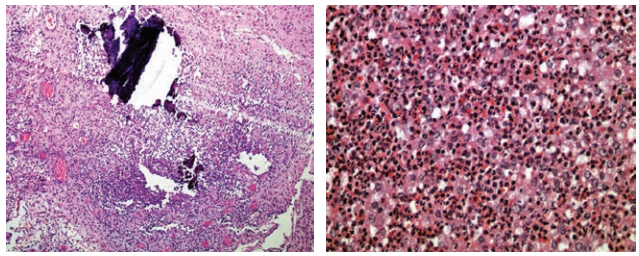


Figure 2A,B. Low and high power examination of biopsy showed sheets of neutrophils.

Discussion

A Brodie abscess is a subacute osteomyelitis which may persist for years before converting to a frank osteomyelitis. Classically, this may present after conversion as a draining abscess extending from the tibia out through the shin. Occasionally acute osteomyelitis may be contained to a localized area and walled off by fibrous and granulation tissue. This is termed as Brodie's abscess.

Brodie's abscess usually presents as a diagnostic dilemma and clinically mimics various benign and malignant conditions such as bone cyst, non-ossifying fibroma, osteoid osteoma, and Ewing sarcoma. Also, sometimes the radiological features are misleading. Typically these present in children, more frequently in boys. It usually occurs at the metaphysis of long bones, distal tibia, proximal tibia, distal femur, proximal or distal fibula, and distal radius. Most frequent causative organism is Staphylococcus aureus. Treatment is mainly surgery. If cavity is small then surgical evacuation & curettage is performed under antibiotic cover. If cavity is large then after evacuation, packing with cancellous bone chips.

Hepatitis C- Frequently asked Questions

Sibtain Ahmed
Section of Clinical Chemistry

- **Is it possible for someone to become infected with HCV and then spontaneously clear the infection?**

  Yes. Approximately 15%–25% of persons clear the virus from their bodies without treatment and do not develop chronic infection; the reasons for this are not well known.

- **How likely is HCV infection to become chronic?**

  HCV infection becomes chronic in approximately 75%–85% of cases.

- **Can persons become infected with a different strain of HCV after they have cleared the initial infection?**

  Yes. Prior infection with HCV does not protect against later infection with the same or different genotypes of the virus. This is because persons infected with HCV typically have an ineffective immune response due to changes in the virus during infection. For the same reason, no effective pre- or post-exposure prophylaxis (i.e., immune globulin) is available.

- **Is there a hepatitis C vaccine?**

  No vaccine for hepatitis C is available. Research into the development of a vaccine is under way

- **How soon after exposure to HCV do symptoms appear?**

  In those persons who do develop symptoms, the average time period from exposure to symptom onset is 4–12 weeks (range: 2–24 weeks).

- **How soon after exposure to HCV can anti-HCV be detected?**

  HCV infection can be detected by anti-HCV screening tests (enzyme immunoassay) 4–10 weeks after infection. Anti-HCV can be detected in >97% of persons by 6 months after exposure.

- **How soon after exposure to HCV can HCV RNA be detected by PCR?**

  HCV RNA appears in blood and can be detected as early as 2–3 weeks after infection.

- **Under what circumstances is a false-positive anti-HCV test result likely?**

  False-positive anti-HCV tests appear more often when persons at low risk for HCV infection (e.g., blood donors) are tested. Therefore, it is important to follow-up all positive anti-HCV tests with a RNA test to establish current infection.

- **Under what circumstances might a false-negative anti-HCV test result occur?**

  Persons with early HCV infection might not yet have developed antibody levels high enough that the test can measure. In addition, some persons might lack the (immune) response necessary for the test to work well. In these persons, further testing such as PCR for HCV RNA may be considered.

- **Can a patient have a normal liver enzyme (e.g., ALT) level and still have chronic hepatitis C?**

  Yes. It is common for patients with chronic hepatitis C to have liver enzyme levels that go up and down, with periodic returns to normal or near normal levels. Liver enzyme levels can remain normal for over a year despite chronic liver disease.

- **Should HCV-infected persons be restricted from working in certain occupations or settings?**

  CDC's recommendations for prevention and control of HCV infection specify that persons should not be excluded from work, school, play, child care, or other settings on the basis of their HCV infection status. There is no evidence of HCV transmission from food handlers, teachers,

or other service providers in the absence of blood-to-blood contact.

- **Should a woman with HCV infection be advised against breastfeeding?**

  No. There is no evidence that breastfeeding spreads HCV. However, HCV-positive mothers should consider abstaining from breastfeeding if their nipples are cracked or bleeding.

- **When should children born to HCV-infected mothers be tested to see if they were infected at birth?**

  Children should be tested for anti-HCV no sooner than age 18 months because anti-HCV from the mother might last until this age. If diagnosis is desired before the child turns 18 months, testing for HCV RNA could be performed at or after the infant's first well-child visit at age 1–2 months. HCV RNA testing should then be repeated at a subsequent visit, independent of the initial HCV RNA test result.

Reference: CDC - Hepatitis C FAQs for Health Professionals; URL: https://www.cdc.gov/hepatitis/hcv/hcvfaq.htm)

Details Of Therapeutic Drug Monitoring Of Antibiotics

Dr Syed Bilal Hashmi, Chemical Pathology

Drug	Time of Sample Collection	Clinical Inofrmation and Uses	Therapeutic Ranges	Toxicity Signs and Symptoms
Gentamicin 	**Peak testing:** Exactly 1 h after start of infusion of 3rd dose, or 1st dose in critically ill patients **Trough:** predose	• Gentamicin is an antibiotic used to treat life-threatening blood infections caused by gram-negative bacilli, particularly Citrobacter freundii , Acinetobacter species, Enterobacter species, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis , Providencia stuartii, Pseudomonas aeruginsa, and Serratia species. • Gentamicin levels are tested for monitoring adequacy of drug clearance during gentamicin therapy • Goal levels depend on the type of infection being treated.	**Adults Multiple daily dose** **Peak:** 4-10 ug/ml **Trough:** 1-2 ug/ml **Adult Once daily dose** **Trough** <1 ug/ml **Pediatrics trough conc.:** <2ug/ml	• Dizziness • Vertigo • Ataxia • Meniere disease-like syndrome • Nephrotoxicity.
Vancomycin 	**Trough** vancomycin concentrations are the most accurate and practical method to guide Vancomycin dosing. Serum trough concentrations should be obtained prior to the fourth or fifth dose	• Vancomycin is an antibiotic used to treat infections caused by gram-positive organisms that are resistant to beta-lactam antibiotics, such as methicillin-resistant staphylococci (MRSA), Staphylococcus viridans group, penicillin/cephalosporin-resistant Streptococcus pneumoniae, and penicillin/ampicillin-resistant Enterococcus species. • Gentamicin levels are tested for monitoring adequacy of drug concentration during vancomycin therapy	**Therapeutic concentration:** >10 ug/ml **Trough:** 15-20 ug/ml	• Nephrotoxicity • Ototoxicity
Amikacin 	**Peak:** Exactly 1 h after start of infusion of 3rd dose, or 1st dose in critically ill patients) **Trough:** Predose	• Amikacin is an aminoglycoside used to treat severe blood infections by susceptible strains of gram-negative bacteria. • Amikacin levels are tested for monitoring adequacy of blood concentration during Amikacin therapy	**Amikacin-Once daily dose** **Trough:** <1.0 ug/ml **Peak:** 56-64 ug/ml **Amikacin-Multiple daily dose** **Trough:** 5-10 ug/ml **Peak:** 15-30 ug/ml	• Ototoxicity, • Nephrotoxicity

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